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Metal Anticancer Compounds

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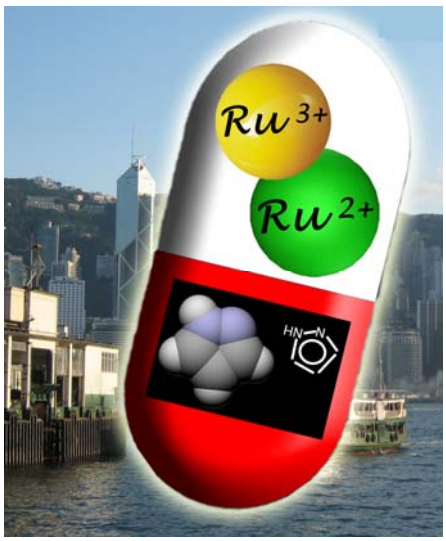

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Gadolinium texaphyrin (Gd-Tex)-malonato-platinum conjugates: Synthesis and comparison with carboplatin in normal and Pt-resistant cell lines

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The synthesis of a new PEG-solubilized gadolinium texaphyrin (Gd-Tex) conjugate containing a malonate-Pt$(NH_3)_2$ moiety is described. The effect of the tumor localizing Gd-Tex macrocycle on platinum activity was evaluated in cell culture. The malonate moiety, analogous to that present in carboplatin, is expected to release an aquated Pt$(NH_3)_2$ species under physiological conditions. The half-life in phosphate-buffered saline was found to be *ca.* 3 days at room temperature, and the hydrolytic product released from the conjugate was collected and confirmed as Pt-based by flameless atomic absorption spectrophotometry. Anti-proliferative activity was tested using A549 human lung cancer and A2780 human ovarian cancer cell lines. In both cell lines, the activity of the Gd-Tex conjugate was found to be similar to that of carboplatin. Efficacy against a Pt-resistant ovarian cell line greater than that displayed by carboplatin was also observed.

Introduction

Cisplatin, carboplatin, and oxaliplatin (Fig. 1) are widely used anti-cancer therapeutic agents. However, these agents also give rise to systemic toxicity as the result, at least in part, of a lack of tumor-specific tissue distribution. Many strategies have been used to increase the tumor specificity of platinum drugs, including conjugation to potentially site-directing molecules, such as folate, poly(ethyleneglycol) (PEG), porphyrins, and peptides among others.[1-5] This work, which has been carried out within the context of a general effort to improve the performance of platinum drugs (by, *e.g.*, delivering active forms of Pt specifically to tumor cells and overcoming identified resistance mechanisms), to our knowledge, has yet to produce an FDA-approved conjugate.

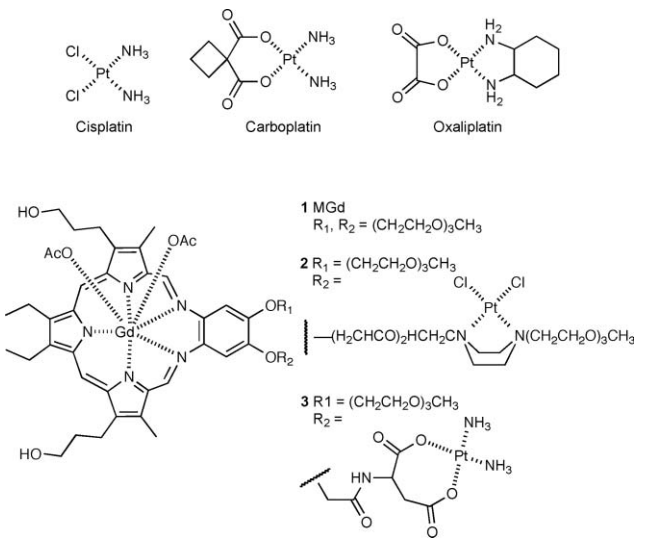

Fig. 1 Structures of cisplatin, carboplatin, oxaliplatin, motexafin gadolinium, MGd **1**, Gd-Tex-amine-Pt conjugate **2**, Gd-Tex-aminosuccinate-Pt conjugate **3**.

In previous work, we have described the synthesis of platinum conjugates based on the structure of motexafin gadolinium (MGd, **1**).[6-9] These were designed to exploit the tumor-localizing properties of this well-studied expanded porphyrin derivative. Unfortunately, the resulting conjugates, which rely on either diamino (**2**) or aminosuccinate (**3**) groups to affect Pt coordination, could not be studied extensively *in vitro* due to a combination of poor solubility and inherent instability (Fig. 1).[7]

The texaphyrins are members of the "expanded porphyrin" class of macrocycles. These pentaaza analogues of porphyrins allow for the coordination of larger metal cations and have led to the development of several well-tolerated experimental therapeutic agents.[6] One of these, motexafin gadolinium, has been the subject of several clinical trials, in which tumor localization was demonstrated by MRI.[10-14]

The inorganic complex cisplatin, and its two FDA-approved analogues, carboplatin and oxaliplatin, are DNA-modifying agents that form interstrand and intrastrand crosslinks thus acting as inhibitors of both bacterial and tumor growth.[15-17] Extensive study has elaborated mechanisms of action, profiles, specificity, applications, and patterns of resistance for these three drugs.[15,16,18-20] As a general rule, therapeutic activity can be correlated with hydrolytic loss of the non-nitrogen ligands (chloride or carboxyl), giving rise initially to a mono-aquated species, which upon completion of hydrolysis, generates the reactive DNA-binding "diaquo" species.[21-26] This rate of hydrolysis, which has been extensively studied, is affected by pH, chloride ion concentration in the case of cisplatin, and the presence or absence of other nucleophiles.[27-30]

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A number of conjugates involving platinum have been prepared in an effort to enhance the therapeutic utility of cisplatin and its analogues. Some archetypal Pt(II) conjugates are represented by non-labile diamino systems, such as **2**, amidosuccinate (aspartate) complexes, represented by **3**, and amino/amidomalonate derivatives, such as **4**[31] and **5** (Fig. 2). In the case of conjugate **2**, attachment was affected *via* piperazine, whereas in the case of conjugate **3**, an aminosuccinato-platinum bonding interaction was employed. While synthetically accessible, these systems demonstrated poor solubility and low activity when subject to *in vitro* biological testing.[7] In the case of the aminosuccinate species **4**, its poor performance was also ascribed to possible premature Pt release. High lability also plagues platinum aminomalonate conjugates in that they are reported to rearrange, giving amine-bound (Pt-N) platinum species. Separately, these compounds demonstrated poor anticancer activity.[31-34] Amidomalonate conjugates, such as **5**, were susceptible to rapid hydrolysis ($t_{1/2}$ = 1 hour) thus limiting therapeutic utility.[35]

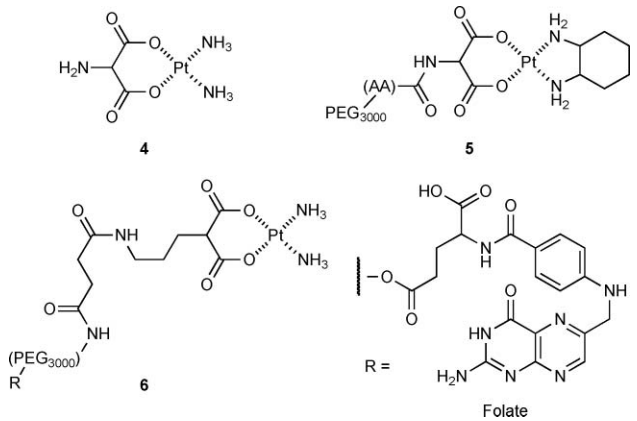


Fig. 2 Malonate-type Pt conjugates: aminomalonate **4**, PEG-amidomalonate **5**, and folate-PEG **6**. AA—amino acid.

Tissue-targeted conjugates have been recently developed. Examples include Pt(II)-PEG conjugates containing both an amino acid linker (AA) and a nuclear localizing sequence (NLS),[36] folate containing complex **6**,[1] in addition to other conjugates containing alkanes, long-chain PEG, steroids, and DNA intercalators.[37,38] Within conjugate **6**, the active species are separated by a PEG linkage in addition to a propyl spacer separating the malonate moeity and the nearby amide. This conjugate displayed increased hydrolytic stability relative to **5** in addition to increased Pt uptake in vitro cell studies in the M109 HiFR cells as determined through a Pt assay.[36] Efficacy evaluation of carboplatin and PEG solubilized conjugate **6** gave IC_{50} values of 18.5 and 16.1 μM, respectively, as inferred from methylene blue proliferation assays. A common factor of these and other active compounds, such as oxaliplatin and the "Platinum Blues"[39] is Pt-dicarboxylate coordination. We thus considered that the incorporation of such a Pt-complexing feature would be advantageous in the construction of improved platinum-gadolinium texaphyrin (Gd-Tex) conjugates. In this report, we detail the synthesis of a new gadolinium texaphyrinmalonate-Pt conjugate, complex **7** (Scheme 1), and show that its antiproliferative activity *in vitro* is comparable to that of carboplatin, as determined from cell proliferation assays involving the A549 human lung cancer and A2780 human ovarian cell lines. We also demonstrate that this conjugate displays higher *in vitro* activity than carboplatin in the Pt-resistant 2780CP ovarian cell line.

Experimental

General procedures

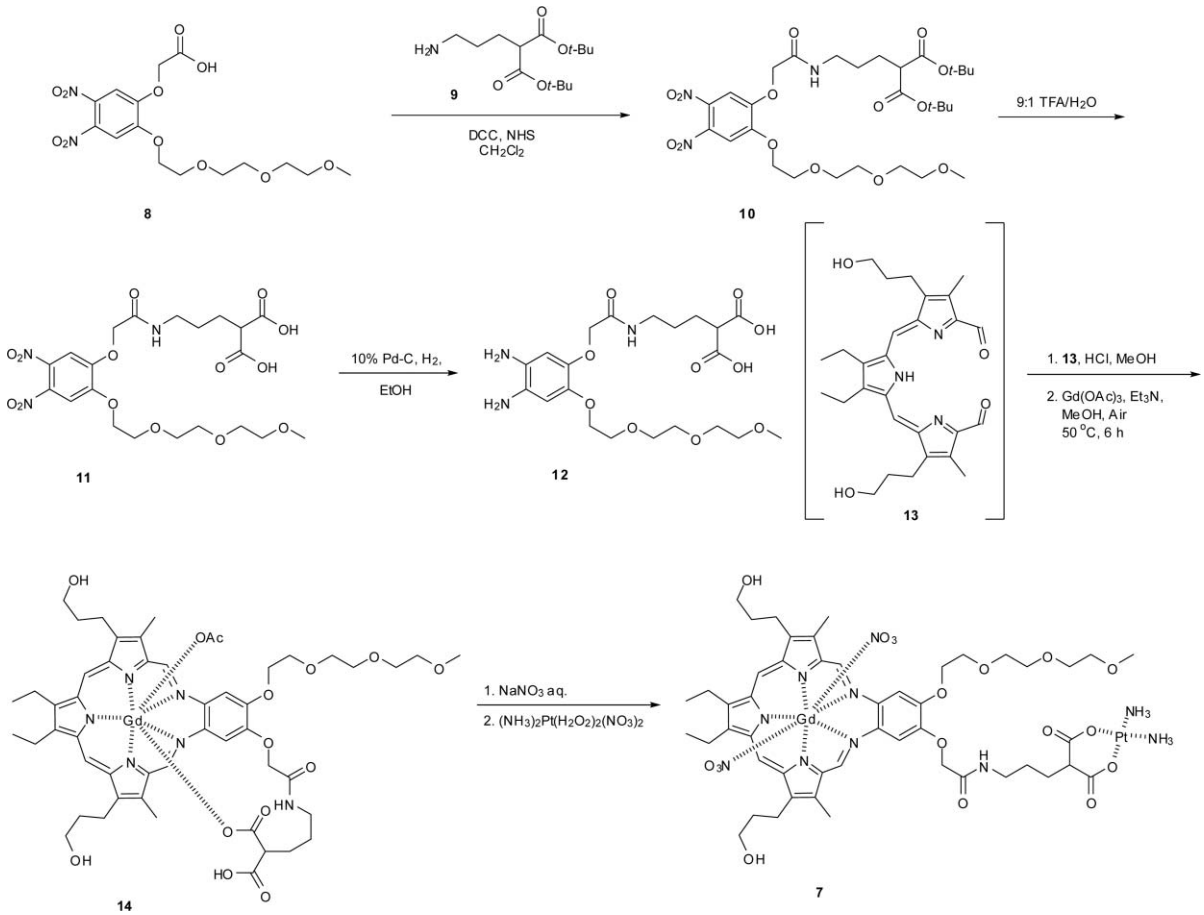
Anhydrous solvents and starting materials were purchased by Fisher Scientific and used without further purification unless otherwise specified. Solvents were dried over molecular sieves, and dichloromethane (DCM) was dried over CaH_2. Intermediate 2,5-bis[(5-formyl-3-hydroxypropyl-4-methylpyrrol-2-yl)methyl]-3,4-diethylpyrrole **13** was supplied by Pharmacyclics, Inc.[40] The starting $Gd(OAc)_3{\cdot}4H_2O$ and $(NH_3)_2PtI_2$ were obtained from Strem Chemical Co. and Johnson Matthey Biomedical Materials, respectively.

Column chromatography was performed with Merck type 60 (230-400 mesh) silica gel using 9:1 DCM:MeOH containing either HOAc or NH_3 to deactivate the silica gel unless otherwise indicated. Sep-Pak cartridges were purchased from Waters or Fisher in 0.5-20 gram sizes of C-18, alumina, or silica gel. Thin layer chromatography (TLC) was performed using Whatman K6F aluminium backed silica gel plates, plastic backed alumina plates, or glass backed C-18 plates. Silica gel TLC of organic soluble samples commonly used EtOAc/hexanes or DCM as the eluents. The conjugates, both before and after treatment with Pt(II), were generally purified on RP-tC18 SPE (Waters Sep-Pak) columns.

HPLC analyses were performed on a Beckman System Gold or Shimadzu Analytical/Preparative HPLC system equipped with PDA detector and a Zorbax XDB 5 μM 5 × 100 mm column. An ammonium acetate buffer (30 mM, pH = 4.3)/acetonitrile (MeCN) gradient (30-99% MeCN over 20 minutes) was used for the analysis of the initial Gd-Tex product. A 0.1% TFA/MeCN gradient (10-99% over 15 minutes unless otherwise noted) was used for analysis of other intermediates. The MGd-Pt conjugate **7** was subject to HPLC analysis on a C-18 column using 0.1% TFA and MeCN as the eluent and detection at 472 nm.

To study the nature of the product hydrolyzed from conjugate **7**, 1.2 mg of **7** was dissolved in 6 mL of PBS and incubated at room temperature for 4 days. At intervals of 24 h, 200 μL aliquots were taken and placed on an RP-tC18 SPE (Waters Sep-Pak) column. The column was then eluted with 4 mL of PBS and the texaphyrin-free eluent collected in 4 × 1mL fractions. The fractions were analyzed for Pt content by flameless atomic absorption spectrophotometry (FAAS), as previously described.[41] Under the conditions used, the standard curve using a certified 1 mg Pt/ml stock solution (Sigma) was linear up to 2 μg/ml, with an r^2 of 0.998.

All ^1H, and ^{13}C NMR spectra were measured using a Varian Mercury 400 (400 MHz) or a Varian Inova 500 (500 MHz) spectrometer using $CDCl_3$, D_2O, CD_3OD or dimethylformamide-d_7 (DMF-d_7) as the solvents. The NMR chemical shifts are reported in ppm relative to the solvent. Low resolution and high resolution electrospray mass spectrometry (ESI MS) were performed using a Thermo Finnigan LTQ instrument and a Qq-FTICR (7 Tesla) instrument, respectively. Mass spectrometry (LRMS, HRMS) was carried out by the University of Texas at Austin Mass Spectrometry Facility. Elemental analyses were



Scheme 1 Synthesis of Gd-Tex-propylmalonate-Pt conjugate **7**.

performed by Midwest Microlabs Inc. Electronic spectra were recorded on a Beckman DU-7 spectrometer.

Synthesis

2-[2-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}-4,5-dinitro-phenoxy)-acetylamino]-malonic acid di-t-butyl ester 10. To a solution of 5.6 g (14 mmol) of acid **8**[7] in 200 mL of DCM containing 1.65 g (14 mmol) of *N*-hydroxysuccinimide (NHS) was added 3.6 g (17 mmol) of dicyclohexylcarbodiimide (DCC). The resulting solution was then stirred briefly. To the solution, 3.7 g (14 mmol) of amine **9**[1] was added. The solution was allowed to stir overnight, filtered, and the solvent was evaporated off. The crude residue was purified by flash chromatography over silica gel (EtOAc:hexanes 1:1, eluent) followed by co-evaporation with $CHCl_3$ to provide 3.2 g (4.9 mmol, 35%) of **10** as an oil. HPLC (monitoring at 329 nm): Tr 14.87 min, 91.24%. TLC (9:1 DCM: MeOH): $R_f = 0.2$. MS (EI) $[M+H]^+ = 660$. HRMS(EI) calculated for $C_{29}H_{46}N_3O_{14}$ 660.2980, found 660.2978. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 18 H), 1.60 (m, 2H), 1.81 (m, 2 H), 3.13 (t, J = 7.2 Hz, 1 H), 3.36 (m, 5 H), 3.53 (m, 2 H), 3.53 (m, 2 H), 3.61-3.67 (m, 4 H), 3.72 (m, 2 H), 3.93 (m, 2 H), 4.37 (m, 2 H), 4.62 (s, 2 H), 7.00 (br t, 1 H), 7.52 (s, 1 H), 7.52 (s, 1 H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.6, 26.9, 2707, 38.6, 53.2, 58.8, 68.8, 69.2, 69.7, 70.3, 70.4, 70.7, 81.4, 109.2, 110.2, 136.0, 137.8, 149.4, 151.9, 166.2, 168.5.

2-(3-(2-(2-(2-(2-(2-Methoxyethoxy)ethoxy)ethoxy)-4,5-dinitro-phenoxy) ethanamido)propyl)propanedioic acid, 11. To 1.7 g (2.6 mmol) of di-*t*-butyl malonate **10** was added 15 mL of 90% v/v TFA in H_2O. The resulting solution was stirred for 1 h and was monitored by TLC and ^{1}H NMR spectroscopic analysis. Once starting material **10** was no longer visible by these techniques, the solvent was removed under reduced pressure. The resulting pellet was triturated with 50 mL $CHCl_3$ and evaporated to dryness under reduced pressure. The product was carried forward without further purification.

Gadolinium(III) complex of 4,5-diethyl-16-acetoxycarbamoyl-3-propyl-[propanediato]-17-(2-[2-(2-methoxyethoxy)ethoxy]ethoxy)-9,24-bis(3-hydroxypropyl)-10,23-dimethyl-13,20,25,26,27-penta-azapentacyclo[20.2.1.1^{3,6}.1^{8,11}.0^{14,19}]heptacosa - 1,3,5,7,9,11(27),12, 14,16,18,20,22(25),23-tridecaene 14. The di-acid **11** obtained as above was dissolved in 50 mL of MeOH containing 0.42 g of 10% Pd-C. The resulting suspension was subjected to high-pressure hydrogenation (40 psi) for 18 h. The suspension was then passed through Celite. The filtrate was then added to a solution consisting of 50 mL of MeOH, 0.5 mL of 3 N aq. HCl, and 1.3 g (2.7 mmol) of tripyrrane **13**.[40] The solution was then stirred for 3 h in a 50 °C oil bath, and monitored by TLC. Once the reaction was deemed complete by TLC analysis, the solvent was evaporated under reduced pressure. To the resulting residue was added 100 mL MeOH, 4 mL of Et_3N, and 1.1 g (2.7 mmol, 1.05

equiv.) of $Gd(OAc)_3 \cdot 4H_2O$. The solution was then stirred to the open atmosphere for 2 h in a 50 °C oil bath. The reaction was then deemed complete by TLC and 4 mL of HOAc was added dropwise. Volatiles were removed under reduced pressure, and the resulting residue was dissolved in 10 mL of 30 mM NH_4OAc (pH 4.3) and loaded on a 10 g C-18 SPE cartridge. Elution with 80:20 MeCN: 30 mM NH_4OAc (pH 4.3) gave fractions rich in the product. These fractions were diluted with NH_4OAc (pH 4.3) and loaded onto a new 10 g C-18 SPE cartridge. The column was washed with water and product **14** eluted with MeOH. Evaporation of the product-containing fractions and titration with Et_2O gave 0.18 g (0.16 mmol, 6.2%) of **14** as a green solid. HPLC (monitoring at 472 nm): Tr 4.28 min 96.24%. MS (EI) $[M - 2\ OAc]^+$ 1084. HRMS(EI) calculated for $C_{49}H_{62}GdN_6O_{12}$ 1084.3667, found 1084.4425. Anal. Calcd for $C_{51}H_{65}GdN_6O_{14}$: C 53.57%; H 5.73%; N 7.35%. Found: C 53.07%; H 5.73%; N 7.55%.

Gadolinium(III) complex of 4,5-diethyl-16-acetoxycarbamoyl-3-propyl-[propanediato-(2-)-O,O′]-[diamine]platinum(II)-17-(2-[2-(2-methoxyethoxy)ethoxy]ethoxy)-9,24-bis(3-hydroxypropyl)-10,23-dimethyl-13,20,25,26,27-pentaazapentacyclo[20.2.1.1^{3,6}.1^{8,11}.0^{14,19}]-heptacosa-1,3,5,7,9,11(27),12,14,16,18,20,22(25),23-tridecaene 7. To a solution of 230 mg (0.201 mmol) of **14** in 10 mL of MeOH was added 40 mL of 100 mM aq. $NaNO_3$, and the resulting suspension was added to a 10 g C-18 SPE cartridge. The nitrated form of **14** was eluted with 15 mL of MeOH. To the methanolic fractions was added 8.0 mL of a 50 mM solution of aq. $(NH_3)_2Pt(H_2O)_2(NO_3)_2$.[42] The resulting solution was stirred in the dark for 48 h. The solution was diluted with an equal volume of 40 mM aq. $NaNO_3$and loaded on a 10 g C-18 SPE cartridge. Elution with 60% v/v 40 mM aq. KNO_3/MeOH effected removal of various unidentified impurities. Conjugate **7** was then eluted from the column using with 40% v/v 40 mM aq. KNO_3/MeOH. The fractions containing conjugate **7** were desalted by diluting 1:1 with 40 mM aq. KNO_3, applying to a new C-18 SPE cartridge, washing with 10 mM aq HNO_3, and eluting with MeOH containing 5% HNO_3 (10 mM, aqueous). Addition of Et_2O precipitated product **7**, which was collected by centrifugation to give 84 mg (0.06 mmol, 30%) of **7** as a green solid.HPLC (monitoring at 472 nm): Tr 7.36 min 95.97%. MS (EI) $(M - NO_3)^+$ 1374. HRMS(EI) calculated for $C_{49}H_{66}GdN_9O_{15}GdPt$: 1373.3567; found: 1373.3574. UV λ_{max} (log ε): 419 (sh), 474 1.19e5, 742 3.43e4. Anal. Calcd for $C_{49}H_{67}GdN_{10}O_{18}GdPt$: C 40.97%; H 4.70%; N 9.75%. Found: C 40.67%; H 4.81%; N 9.46%.

In vitro anti-proliferative activity

The proliferation of exponential phase cultures of A549, A2780 and 2780CP cells was assessed by tetrazolium salt reduction.[43] In brief, tumor cells were seeded in 96-well microtiter plates at 3000, 300 and 1000 cells/well, respectively, and allowed to adhere overnight in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% heat inactivated fetal bovine serum, and antibiotics (200 U/cm^3 penicillin and 200 μg/cm^3 streptomycin). Stock solutions of carboplatin (5% aqueous mannitol or ultrapure H_2O), MGd (methanol), or conjugate **7** (methanol) were formulated in the indicated solvent for maximum stability and then diluted in medium for secondary stocks of 40-1000 μM depending on the cell line being tested. Secondary stock solutions were serially diluted in medium and immediately added to wells, whereupon plates were incubated at 37 °C under a 5% CO_2/95% air atmosphere. After a total of 3 (A549 cells) or 5 (A2780 or 2780CP cells) days, the tetrazolium dye, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical) was added to each well and the plates incubated at 37 °C, whereupon the medium was removed, the formazan dissolved and absorbances measured at 560-650 nm using a microplate reader (Molecular Devices, Sunnyvale, CA). Absorbances were corrected for background and the values normalized to wells containing untreated cells to allow plate-to-plate comparison. The data are shown as mean inhibition of proliferation or growth as a % of control cells from 8-10 replicate values. Error bars represent the associated standard deviation.

Results and discussion

Synthesis of Gd-Tex platinum(II) conjugate

The apparent success of Pt conjugate **6** led us to attempt the synthesis of texaphyrin-Pt(II) conjugate **7**, bearing a carboplatin-like coordination site. The synthesis of this new conjugate is shown in Scheme 1. Amide bond formation between acid **8**[7] and amine **9**[1] was accomplished using dicyclohexylcarbodiimide (DCC) in the presence of *N*-hydroxysuccinimide (NHS). The resulting dinitrophenyl malonate diester **10** was then treated with 9:1 TFA/H_2O, employing conditions utilized in peptide deprotection;[44] this effected deprotection of the acid groups, giving diacid **11**. Although removal of the malonate *t*-butyl groups was expected to be facile, we found that more traditional conditions, such as treatment with neat TFA, formic acid, or TMS-triflate, were not effective.[45,46] Dinitro **11** was then converted to diamine **12** by catalytic reduction. Cyclization between equimolar amounts of diamine **12** and dialdehyde **13** followed by oxidative metallation with $Gd(OAc)_3$ provided Gd coordinated texaphyrin **14** as the dominant product (6.2% yield based on **10**). Analysis of the crude mixture indicated less than 20% formation of the mono-decarboxylation biproduct. These two products were separated by solid phase extraction (SPE) on C-18 using acetonitrile and ammonium acetate buffer. The resulting purified texaphyrin, a paramagnetic complex, was then characterized by elemental analysis, HPLC, and mass spectrometry.

With the Gd-Tex-derived malonate complex **14** in hand, efforts were made to affect platination of the malonic acid moiety. Initial attempts using $(NH_3)_2Pt(H_2O)_2 \cdot (NO_3)_2$ (hereafter Pt-diaquo) as a platinum(II) source proved unsuccessful. Literature reports[30] detailing the reaction of Pt-diaquo species with acetate, led us to consider that the acetate counter anion present in **14** might be interfering with this complexation process. Complex **14** was thus subject to nitrate-for-acetate ion exchange by loading **14** on a C-18 Sep-Pak column and eluting with $NaNO_3$ buffer to remove the NH_4OAc buffer used in the initial purification. Removal of ammonia (presumably in its protonated form) and acetate, provided the nitrated species suitable for coordination to platinum.[30]

A methanol solution of the resulting texaphyrin (nitrate) complex analogous to **14** was mixed with an aqueous solution containing one equivalent of diaquo Pt complex and left to stir for 24 hours. Quenching the reaction by pouring into water gave a green solid in 30% yield (based on **14**). Analysis by UV

spectroscopy, LCMS, and elemental analysis provided support for this species being the desired platinated-texaphyrin conjugate **7**. As determined *via* HPLC studies, platinated Gd-Tex-based **7** eluted faster than the starting texaphyrin-diacid **14**. Final purification was thus affected on a reverse phase C-18 column using a methanol/KNO_3 mobile phase. Desalting was also effected on a reverse phase C-18 column by washing the bound material with water prior to elution with methanol. This produced product **7**, a paramagnetic species, which was characterized by HPLC, mass spectrometry, and elemental analysis.It was this material that was tested in the cell proliferation studies (*vide infra*). Due to the distance between the paramagnetic Gd texaphyrin center and the Pt center, it was thought that characterization of the Pt coordination environment and the organic framework of the proposed chelates might be possible using ^{1}H, ^{13}C, and ^{195}PtNMR. These studies were attempted; however, presumably as a consequence of the paramagnetism of the Gd center, the peaks obtained were either too broad for characterization or simply not visible. However, the structure given for **7** is consistent with all other available evidence. Moreover, an alternative formulation, involving N-Pt-O coordination rather than O-Pt-O ligation, was ruled out on the basis of the larger chelate ring that would need to be formed and the slower hydrolysis rate expected for an N-Pt, as compared to O-Pt linkage.

As anticipated given the O-Pt-O coordination environment present in conjugate **7**, aqueous solutions of this complex were found to hydrolyze to produce $Pt(NH_3)_2$ species and organic fragments over time, as inferred from HPLC and FAAS analysis. Initial HPLC analysis of aqueous solutions of the platinated-Gd-Tex complex **7** revealed decreasing levels of the platinated material and increasing levels of the Gd-Tex-diacid **14** as a function of time. However, this proved to be a reversible process; the HPLC trace corresponding to **7** could be regenerated by the addition of excess Pt-diaquo (data not shown). Further, conjugate **7** proved to be stable both in the presence of high concentrations of nitrate (0.5-1.0 M, aq.) and in methanol solution.

Stability studies of Gd-Tex-Pt conjugate 7

Given the reversible loss of Pt seen during the purification of Gd-Tex-Pt conjugate **7**, efforts were made to quantify the rate of this presumed hydrolytic process. Towards this end, stability studies were carried out in two different media using HPLC (Fig. 3). These studies revealed that the half-life of platinum loss for conjugate **7** in phosphate-buffered saline is *ca.* 72 h, a value that is commensurate with the rate of carboplatin hydrolysis.[24]

As expected, the conjugate proved much more stable in methanol than in aqueous medium (Fig. 3a).

The hydrolysis of conjugate **7** was further monitored by FAAS (Fig. 3b). Conjugate **7** was dissolved in PBS buffer and incubated for 4 days. Aliquots were sampled immediately and at 24 h intervals. They were processed using RP-tC18 SPE columns with PBS as the eluent. The colored texaphyrin complex was retained on the column, under conditions where the more polar hydrolysed Pt species is eluted. The collected 1 mL fractions were subjected to FAAS for determination of Pt concentration. The Pt-containing hydrolysis product(s) formed from **7** was initially negligible, but became measurable within 24 hours and continued to form thereafter. Pt elution was almost complete using 4 mL

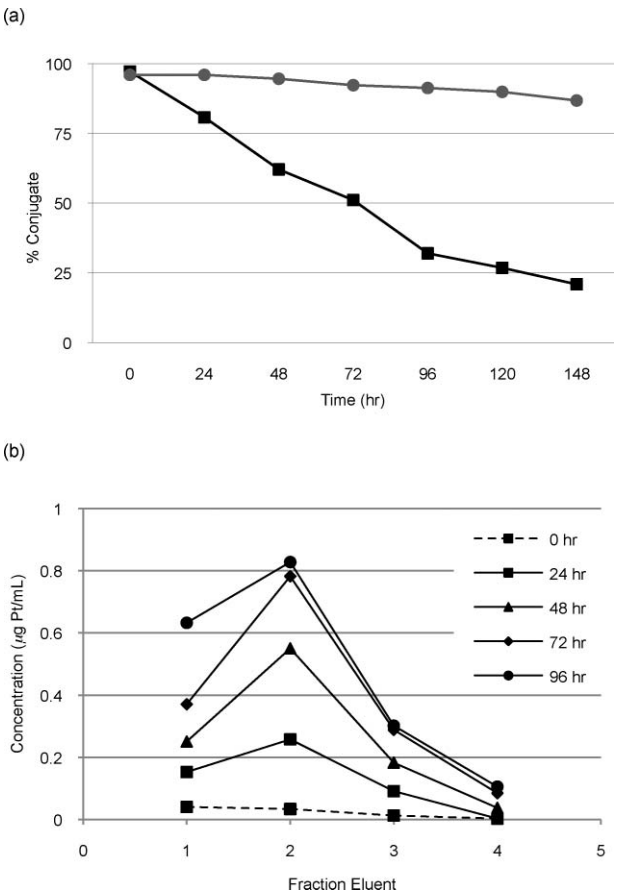


Fig. 3 (a) HPLC hydrolysis study of Gd-Tex-Pt conjugate **7** in methanol (grey line) and phosphate buffered saline (PBS) (black line). Samples in PBS display a $t_{1/2}$ of *ca.* 3 days. (b) Pt concentration in eluates collected from a Sep Pak column pre-loaded with an aliquot of conjugate **7** in PBS incubated over 4 days. Each fraction represents 1 mL of eluent.

PBS for elution, as indicated by a low Pt concentration in the fourth fraction at each time-point. Due to the lack of retention on the column, the Pt is most likely inorganic in nature and may consist of cisplatin due to the high chloride concentration in the PBS buffer.

Antiproliferative activity of Gd-Tex-Pt conjugate 7

Initial tests for antiproliferative activity were carried out using the A549 human lung cancer cell line. These studies revealed that conjugate **7** had a low level of efficacy, but one that was similar to carboplatin or an equimolar combination of MGd and carboplatin (Fig. 4). MGd alone had no effect on A549 cell proliferation. This finding, coupled with the duration of the treatment (3 days) and the measured half-life of Pt release from **7** (about 3 days, Fig. 3), is consistent with the notion that conjugate **7** undergoes hydrolysis in tissue culture medium at a rate similar to that of carboplatin. It also led us to consider whether conjugate **7** could better be evaluated in more sensitive cell lines where both Pt-based activity and resistance had previously been observed.[47]

With such considerations in mind, tests were carried out using the A2780 human ovarian cancer cell line and an ostensibly isogenic cisplatin-resistant cell line (2780CP) (Fig. 5). As before, the effect of conjugate **7** on cell proliferation was compared to that

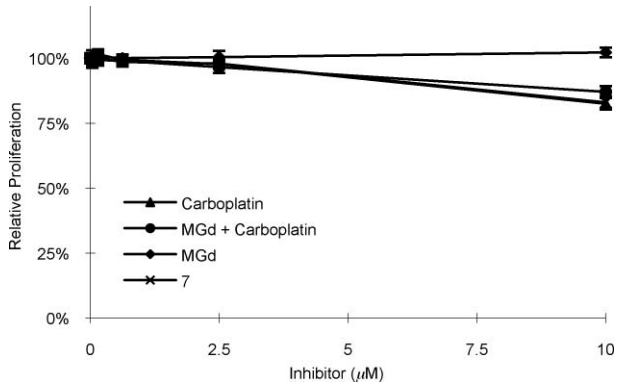


Fig. 4 Effect of conjugate **7** on cell proliferation. A549 lung cancer cells were incubated with inhibitors for 3 days at 37 °C in 5% CO_2 and relative proliferation measured by tetrazolium salt reduction. The anti-proliferative activity of MGd, carboplatin, or the combined treatment is shown for comparison. Error bars represent standard deviation.

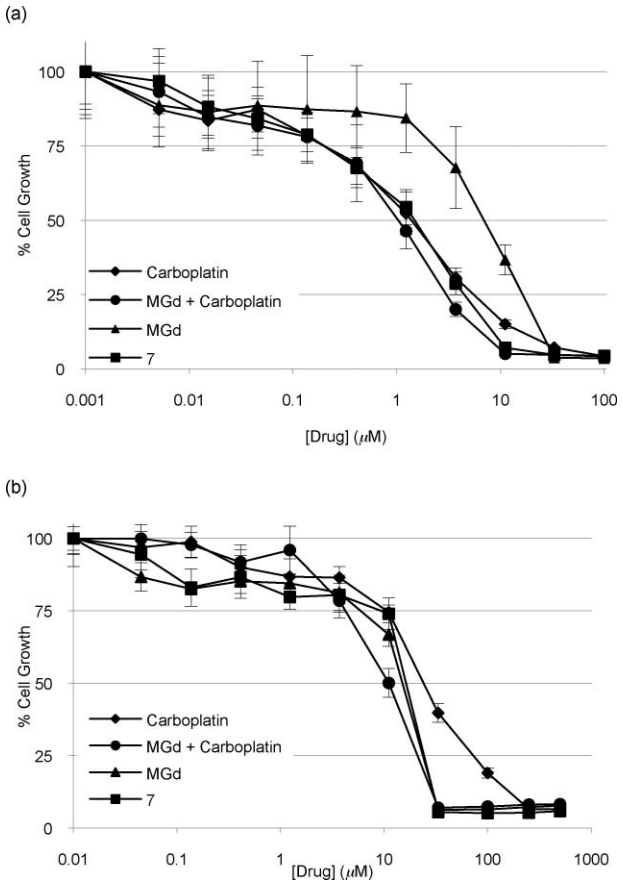


Fig. 5 MTT Cell proliferation assay with A2780 (a) and cisplatin resistant 2780CP (b) ovarian cancer cell lines in the presence of variable concentrations of indicated drug. The drug was added to cells and incubated for 5 days at 37 °C in 5% CO_2. Error bars represent standard deviation.

of equimolar concentrations of carboplatin, MGd, or a mixture of these two compounds. Carboplatin ($IC_{50} = 1.6$ μM) provided a dose-responsive profile that was similar to literature values using this cell line.[47] Conjugate **7** displayed a similar level of activity ($IC_{50} = 1.4$ μM) as carboplatin within error. This similarity in activity is encouraging considering the many known platinum conjugates with reduced activity.[36,48] While it may be inferred from the similarity in IC_{50} values between carboplatin and conjugate **7** that activity may be attributed to platinum delivery, this has yet to be shown experimentally. An antiproliferative effect was also observed using higher concentrations of MGd ($IC_{50} = 6.3$ μM). However, no significant synergistic effects were observed with cells incubated with equimolar amounts of carboplatin and MGd ($IC_{50} = 1.2$ μM).

Cell proliferation studies were also performed with platinum resistant ovarian cancer cells (2780CP). In this instance, cells incubated in the presence of the texaphyrin complex, *i.e.*, treatment with MGd + carboplatin, MGd, or conjugate **7**, were inhibited to a similar degree, with IC_{50} values of 11.6, 13.7, and 14.4 μM, respectively. The activity of carboplatin was lower (26.3 μM), consistent with literature values.[46] The texaphyrin containing treatments provided for a roughly two fold greater effect as compared to treatment with carboplatin alone. While this is an encouraging result, it is important to note that at this time it remains unknown whether the activity of conjugate **7** is attributable to the texaphyrin complex, the active platinum species, or a combination of both. Further studies with additional conjugates are thus planned in an effort to determine the relative contributions of these and other factors (*e.g.*, cell uptake) that can influence cell proliferation.

Conclusions

In attempts to develop a system for the targeted delivery of platinum to cancer cells, conjugate **7**, containing a Gd-Tex and malonate-$Pt(NH_3)_2$ moieties, was designed and synthesized. The tumor localizing Gd-Tex core was intended to act as a delivery vehicle for the coordinated platinum. The malonate chelating group, analogous to that present in carboplatin, was designed to release Pt under physiological conditions. The rate of release in phosphate-buffered saline was found to be *ca.* 3 days at room temperature. Anti-proliferative activity was tested initially using the A549 human lung cancer cell line and then more extensively in the A2780 human ovarian cancer cell line. In both cases the activity was found to be similar to that of carboplatin. Activity against a Pt-resistant ovarian cell line (2780CP) was greater than that displayed by carboplatin. While the activity profiles of conjugate **7** are highly encouraging, it should be noted that the mechanism of action of **7** is not yet known. Possible mechanisms include induced oxidative stress *via* the texaphyrin core, DNA crosslinking *via* the active platinum agent, a combination of both, or an entirely new mechanism of action yet to be elucidated. Further studies with additional conjugates are thus planned in an effort to understand the activity of **7** and related texaphyrin-based platinum species.

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